STUDIES IN THE PHYSIOLOGY OF THE FUNGI

VII. Growth of Wood-Destroying Fungi on Liquid Media

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For some time this laboratory has been conducting many lines of investigation to determine various physiological factors influencing the growth and development of the wood-destroying fungi. In this connection experiments have been undertaken to determine (1) which fungi are adapted to growth on liquid media; (2) what liquid media are suitable to their growth; and (3) the influence, if any, of the hydrogen ion concentrations of the media. To this end various media were used. In three of these (Czapek's, Reed's, and Richards') the H ion concentration was made to vary by using the mono–, di–, and tribasic potassium phosphates in the different solutions.

The solutions used were as follows:

1. *Czapek's solution*[1] which contains 0.5 gm. magnesium sulphate, 1.0 gm. monobasic potassium phosphate, 0.5 gm. potassium chloride, 0.01 gm. ferrous sulphate, 2.0 gms. sodium nitrate, and 30 gms. cane sugar in 1000 cc. of water.

2. *Dunham's solution* made up according to the usual method, using 10 gms. bacto-peptone and 5 gms. sodium chloride in 1000 cc. of water.

3. *A pine decoction* prepared from the inner bark and outer sap-wood of young twigs of *Pinus Strobus*.

4. *Reed's solution*[2] for the determination of cytase, which contains 10 gms. ammonium nitrate, 5 gms. dibasic potassium phosphate, 1 gm. magnesium sulphate, and 1 gm. sodium citrate in 1000 cc. of ash-cellulose suspension in water.[3]

[1] Dox, A. W. The intracellular enzymes of Penicillium and Aspergillus. U. S. Dept. Agr., Bur. An. Ind. Bul. 120: 1–70. 1910.

[2] Reed, H. S. The enzyme activities involved in certain fruit diseases. Va. Polytec. Inst., Agr. Exp. Sta. Rept. 1911-1912: 51–77. 1913.

[3] The method of preparing pure cellulose from ash wood was that described by McBeth and Scales, U. S. Dept. Agr., Bur. Pl. Ind. Bul. 266: 1–52. 1913.

5. *Richards' solution* (*E*)[1] which contains 0.5 gm. monobasic potassium phosphate, 4 gms. potassium nitrate, 2.5 gms. magnesium sulphate, 10 gms. ammonium nitrate, 50 gms. cane sugar, and a trace of ferrous sulphate in 1000 cc. of water.

6. *Sap from Acer saccharinum.*

The following twelve fungi were employed in the experiments: *Coniophora cerebella* Pers., *Daedalea confragosa* (Bolt.) Fr., *D. quercina* (L.) Fr., *Lentinus lepideus* Fr., *Lenzites vialis* Pk., *Merulius lacrymans* (Wulf.) Fr., *M. pinastri* (Fr.) Burt, *Pleurotus sapidus* Kalchb., *Polyporus lucidus* (Leys.) Fr., *Polystictus hirsutus* Fr., *P. versicolor* (L.) Fr., and *Trametes Peckii* Kalchb.

METHODS

Twenty-five-cc. quantities of the above-mentioned solutions and decoctions were pipetted into Erlenmeyer flasks (125 cc. capacity). Duplicates of these were inoculated with each fungus after the flasks were sterilized at 15 pounds pressure for 20 minutes. Control flasks of all solutions were prepared in duplicate.

Before inoculation the fungi were all grown on agar-poured plates. The nutrient agar was made up in the following manner: To 1000 cc. of potato water (from 200 gms. of peeled and sliced potato cooked for 30 minutes in the autoclave at 15 pounds pressure) were added 20 gms. of cane sugar, 10 gms. of potassium nitrate, 5 gms. of monobasic potassium phosphate, and 20 gms. of agar. After growth of the fungi the plates were cut into small squares (about 8 mm. square) which were used as inocula. Thus the amount of agar added to the cultures introduced to all a negligible amount of nutrient materials. Similar squares of agar from uninoculated poured plates were added to all control flasks. All cultural operations were performed in a transfer room which was thoroughly steamed before each operation. There were no contaminations in the 332 cultures involved in the data discussed below.

[1] Richards, H. M. Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. f. wiss. Bot. **30**: 665–688. 1897.

The cultures were incubated at room temperature (about 15–25° C.) during a period of 30 days.

The growth of the fungi which is reported in values of × in the table was determined as follows: The culture of maximum growth in the whole series was arbitrarily designated as 10 ×, no growth as 0, and intermediate amounts of growth as 1 ×, 2 ×, 3 ×, etc.

The H ion concentrations were determined in accordance with the methods devised by Clark and Lubs,[1] and are given in P_H exponents. In the table are given the P_H values of the control flasks, the P_H values of the solutions after the fungi had grown in them for thirty days, and the amount of change in the H ion concentration of the solutions brought about by the growth of the various fungi. If the change due to growth of the organism is toward the alkaline side from the true neutral point (P_H 7) it is designated as minus and if toward the acid side, plus.

Discussion

The data presented in the table bring out some interesting features concerning the comparative growth of the fungi involved. There is a decrease in the amount of growth of *Lenzites vialis, Daedalea confragosa, D. quercina, Trametes Peckii, Merulius lacrymans, Lentinus lepideus, Coniophora cerebella, Polyporus lucidus,* and *Polystictus hirsutus* when passing from the mono- to the tribasic potassium phosphate in Czapek's solution. In *Merulius pinastri* there is a slight increase in growth, in *Pleurotus sapidus* no variation, while the growth of *Polystictus versicolor* is irregular when passing in the same direction.

In Reed's solution there is a decrease in the growth quantities of all of the fungi, except *Pleurotus sapidus, Merulius lacrymans,* and *Coniophora cerebella,* when passing from the mono- to the tribasic potassium phosphate. In *Pleurotus sapidus* growth is equal in all cases, in *Merulius lacrymans* there is a steady increase, while in *Coniophora cerebella* it is irregular.

[1] Clark, W. M., and Lubs, H. A. The colorimetric determination of hydrogen ion concentration and its applications in bacteriology. I. Jour. Bact. **2:** 1–34. 1917.

TABLE I

GROWTH OF WOOD-DESTROYING FUNGI ON LIQUID MEDIA

Fungus		Dunham's Solution	Czapek's sol. (1) with KH_2PO_4	Czapek's sol. (2) with K_2HPO_4	Czapek's sol. (3) with K_3PO_4	Reed's sol. (1) with KH_2PO_4	Reed's sol. (2) with K_2HPO_4	Reed's sol. (3) with K_3PO_4	Maple sap	Richards' sol. (1) with KH_2PO_4	Richards' sol. (2) with K_2HPO_4	Richards' sol. (3) with K_3PO_4	Pine decoction
L. vialis	Gr.*	0	2×	2×	0	3×	2×	0	3×	5×	5×	5×	0
	P_H†	7.0	3.4	6.0	8.6	5.2	6.6	7.0	7.2	2.6	2.6	3.0	5.2
	Diff.‡	0.0	+1.8	+1.0	0.0	0.0	0.0	0.0	0.0	+2.2	+3.2	+3.2	+0.2
M. pinastri	Gr.	4×	9×	9×	10×	3×	2×	1×	8×	3×	5×	8×	0
	P_H	7.6	5.8	7.4	7.4	6.2	6.8	7.0	6.2	5.4	6.8	6.6	5.4
	Diff.	−0.6	−0.6	−0.4	+1.2	−1.0	−0.2	0.0	+1.0	−0.6	−1.0	−0.4	0.0
D. quercina	Gr.	0	6×	0	0	3×	0	0	0	6×	5×	1×	0
	P_H	7.0	3.2	7.0	8.6	5.2	6.6	7.0	7.0	2.6	2.8	5.4	5.4
	Diff.	0.0	+2.0	0.0	0.0	0.0	0.0	0.0	+0.2	+2.2	+3.0	+0.8	0.0
T. Peckii	Gr.	4×	5×	2×	0	3×	2×	0	4×	3×	6×	4×	0
	P_H	7.0	4.8	6.2	8.6	5.6	6.6	2.0	6.0	3.4	3.6	5.2	5.4
	Diff.	0.0	+0.4	+0.8	0.0	−0.4	0.0	0.0	+1.2	+1.4	+2.2	+1.0	0.0
P. sapidus	Gr.	5×	1×	1×	1×	2×	2×	2×	4×	2×	4×	5×	4×
	P_H	7.0	5.0	6.4	8.6	5.2	6.6	7.0	6.6	3.6	3.4	3.4	6.0
	Diff.	0.0	+0.2	+0.6	0.0	0.0	0.0	0.0	+0.6	+0.8	+2.4	+2.8	−0.6
M. lacrymans	Gr.	1×	2×	0	0	2×	3×	4×	1×	4×	5×	6×	0
	P_H	7.0	4.6	2.0	8.6	5.6	6.6	7.0	7.0	2.8	2.8	3.0	5.4
	Diff.	0.0	+0.6	0.0	0.0	0.0	0.0	0.0	+0.2	+2.0	+3.0	+3.2	0.0
L. lepideus	Gr.	0	3×	0	0	1×	0	0	1×	2×	2×	2×	0
	P_H	7.0	4.8	6.8	8.6	5.2	6.6	7.0	7.0	3.2	4.0	5.6	5.4
	Diff.	0.0	+0.4	+0.2	0.0	0.0	0.0	0.0	+0.2	+1.6	+1.8	+0.6	0.0
D. confragosa	Gr.	1×	2×	1×	0	3×	1×	0	3×	1×	2×	3×	0
	P_H	6.8	5.0	6.8	8.4	5.6	6.6	7.0	7.6	3.0	2.8	3.4	5.4
	Diff.	+0.2	+0.2	+0.2	+0.2	−0.4	0.0	0.0	−0.4	+1.8	+3.0	+2.8	0.0
C. cerebella	Gr.	0	1×	0	0	1×	0	1×	0	3×	2×	2×	0
	P_H	7.0	5.2	7.2	8.6	5.4	6.6	7.4	7.4	2.8	3.2	3.4	5.4
	Diff.	0.0	0.0	−0.2	0.0	−0.2	0.0	−0.4	−0.2	+2.0	+2.6	+2.8	0.0
P. versicolor	Gr.	0	3×	5×	2×	5×	2×	1×	7×	3×	3×	4×	5×
	P_H	7.0	4.8	4.8	4.8	6.0	6.8	7.2	5.0	3.0	3.2	3.0	5.8
	Diff.	0.0	+0.4	+2.2	+3.8	−0.8	−0.2	−0.2	+2.2	+1.8	+2.6	+3.2	−0.4
P. lucidus	Gr.	3×	7×	2×	1×	2×	2×	0	4×	4×	5×	6×	6×
	P_H	5.0	4.2	6.6	8.6	5.6	6.6	7.0	4.2	4.2	4.4	5.0	5.2
	Diff.	+2.0	+1.0	+0.4	0.0	−0.4	0.0	0.0	+3.0	+0.6	+1.4	+1.2	+0.2
P. hirsutus	Gr.	0	2×	1×	0	3×	2×	0	4×	1×	2×	3×	5×
	P_H	7.0	4.2	4.4	8.6	5.6	6.6	7.0	4.8	3.2	3.2	3.2	5.2
	Diff.	0.0	+1.0	+2.6	0.0	−0.4	0.0	0.0	+2.4	+1.6	+2.6	+3.0	0.0
Control	P_H	7.0	5.2	7.0	8.6	5.2	6.6	7.0	7.2	4.8	5.8	6.2	5.4

* Gr. = growth; † P_H = H ion concentration; ‡ Diff. = change in H ion concentration due to growth.

There is a decrease in the amount of growth of *Daedalea quercina* and *Coniophora cerebella* when passing from the mono- to the tribasic potassium phosphate in Richards' solution. In *Merulius lacrymans, M. pinastri, Trametes Peckii, Pleurotus sapidus, Daedalea confragosa, Polystictus versicolor, P. hirsutus,* and *Polyporus lucidus* there is an increase, while in *Lenzites vialis* and *Lentinus lepideus* growth is equal in all cases.

In general, Dunham's solution and the pine decoction cannot be considered suitable media for these fungi, while maple sap compares favorably with the best solution (Richards' with the tribasic potassium phosphate).

In all of the fungi studied, except *Merulius pinastri,* the general tendency is to increase the active acidity during growth. However, there are exceptions to the general tendencies, indicating the fallacy of combining the results obtained from several fungi to draw sweeping conclusions as to a definite relation between the H ion concentration and growth of wood-destroying fungi as a group. Thus, we doubt the advisability of constructing a general curve with the average data derived from a limited number of fungi, as Meacham has done in a recent note.[1] This curve would not apply to many wood-destroying fungi, as the data in the table indicate.

The influence of the growth of the same fungi on the P_H value of different solutions is shown by a comparison of the data on Reed's and Richards' solutions. On Reed's solution containing the monobasic potassium phosphate all of the fungi made fair growth and raised slightly the P_H value; on the same solution with the dibasic potassium phosphate all of the fungi made slight growth only, and there is a tendency to raise the P_H value; while with the tribasic potassium phosphate those that grew exhibited a tendency to raise the P_H value. However, on Richards' solution with the monobasic potassium phosphate all of the fungi, with the exception of *Merulius pinastri,* lower even the very low P_H value of the solution; and

[1] Meacham, M. R. Note upon the hydrogen ion concentration necessary to inhibit the growth of four wood-destroying fungi. Science N. S. **48:** 499–500. *f. 1.* 1918.

although the P_H values of the solutions with the di- and tribasic potassium phosphates were originally greater than with the monobasic phosphate the lowering due to growth was very pronounced. This difference in the shifting of the active acidity due to growth in Reed's and Richards' solutions may be attributed to the fact that in Reed's solution the citrate radical of the sodium citrate acts as the source of carbon, thus liberating alkaline sodium compounds.

Conclusions

1. Many wood-destroying fungi are not suitable for growth experiments with liquid media.

2. With respect to the media employed and to the species studied, *Merulius pinastri, Polyporus lucidus, Polystictus versicolor, Pleurotus sapidus,* and *Trametes Peckii* grow best in the order named. Others grow well only on certain media, e. g., *Lenzites vialis, Daedalea quercina,* and *Merulius lacrymans* on Richards' solution.

3. Czapek's solution with the monobasic, and Richards' solution with the mono-, di-, and tribasic potassium phosphate proved generally to be suitable media. Thus, there is a decided indication of the desirability of selecting a specific medium for each fungus.

4. In the solutions studied the H ion concentration does not seem to be the limiting factor in growth, nor in general does it appear to be the factor (within the limits studied) which determines a desirable medium.

5. The shifting of the H ion concentration due to metabolism depends both upon the fungus and the medium.

6. No general statement can be made concerning the relation between the H ion concentrations of the culture media and the growth of wood-destroying fungi as a group.